

Tyrosine-Selective Functionalization for Bio-Orthogonal Cross-Linking of Engineered Protein Hydrogels.

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Public Summary:

Engineered protein hydrogels have shown promise as artificial extracellular matrix materials for the 3D culture of stem cells due to the ability to decouple hydrogel biochemistry and mechanics. The modular design of these proteins allows for incorporation of various bioactive sequences to regulate cellular behavior. However, the chemistry used to cross-link the proteins into hydrogels can limit what bioactive sequences can be incorporated, in order to prevent nonspecific cross-linking within the bioactive region. Bio-orthogonal cross-linking chemistries may allow for the incorporation of any arbitrary bioactive sequence, but site-selective and scalable incorporation of bio-orthogonal reactive groups such as azides that do not rely on commonly used amine-reactive chemistry is often challenging. In response, we have optimized the reaction of an azide-bearing 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) with engineered elastin-like proteins (ELPs) to selectively azide-functionalize tyrosine residues within the proteins. The PTAD-azide functionalized ELPs cross-link with bicyclononyne (BCN) functionalized ELPs via the strain-promoted azide-alkyne cycloaddition (SPAAC) reaction to form hydrogels. Human mesenchymal stem cells and murine neural progenitor cells encapsulated within these hydrogels remain highly viable and maintain their phenotypes in culture. Tyrosine-specific modification may expand the number of bioactive sequences that can be designed into protein-engineered materials by permitting incorporation of lysine-containing sequences without concern for nonspecific cross-linking.

Scientific Abstract:

Engineered protein hydrogels have shown promise as artificial extracellular matrix materials for the 3D culture of stem cells due to the ability to decouple hydrogel biochemistry and mechanics. The modular design of these proteins allows for incorporation of various bioactive sequences to regulate cellular behavior. However, the chemistry used to cross-link the proteins into hydrogels can limit what bioactive sequences can be incorporated, in order to prevent nonspecific cross-linking within the bioactive region. Bio-orthogonal cross-linking chemistries may allow for the incorporation of any arbitrary bioactive sequence, but site-selective and scalable incorporation of bio-orthogonal reactive groups such as azides that do not rely on commonly used amine-reactive chemistry is often challenging. In response, we have optimized the reaction of an azide-bearing 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) with engineered elastin-like proteins (ELPs) to selectively azide-functionalize tyrosine residues within the proteins. The PTAD-azide functionalized ELPs cross-link with bicyclononyne (BCN) functionalized ELPs via the strain-promoted azide-alkyne cycloaddition (SPAAC) reaction to form hydrogels. Human mesenchymal stem cells and murine neural progenitor cells encapsulated within these hydrogels remain highly viable and maintain their phenotypes in culture. Tyrosine-specific modification may expand the number of bioactive sequences that can be designed into protein-engineered materials by permitting incorporation of lysine-containing sequences without concern for nonspecific cross-linking.

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